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Role of dopamine D₂-like receptors and their modulation by adenosine receptor stimulation in the reinstatement of methamphetamine seeking

Tracey A. Larson[#], Madeline C. Winkler[#], Jacob Stafford, Sophia C. Levis, Casey E. O'Neill, and Ryan K. Bachtell

Department of Psychology and Neuroscience and Center for Neuroscience, University of Colorado Boulder, Boulder, CO, USA

[#] These authors contributed equally to this work.

Abstract

Rationale and objective: Previous work has demonstrated that dopamine and adenosine receptors are involved in drug seeking behaviors, yet the pharmacological interactions between these receptors in methamphetamine (MA) seeking **are** not well characterized. The present studies examined the role of the dopamine D₂-like receptors in MA seeking and identified the interactive effects of adenosine receptor stimulation.

Methods: Adult male Sprague-Dawley rats were trained to lever press for MA in daily 2-hour self-administration sessions on a fixed-ratio 1 schedule for 10 consecutive days. After 1 day of abstinence, lever pressing was extinguished in 6 daily extinction sessions. Treatments were administered systemically prior to a 2-hour reinstatement test session.

Results: An increase in MA seeking was observed following the administration of the dopamine D₂-like agonist, quinpirole, or the D₃ receptor agonist, 7-OH-DPAT. Stimulation of D₂ or D₄ receptors was ineffective at inducing MA seeking. Quinpirole-induced MA seeking was inhibited by D₃ receptor antagonism (SB-77011A or PG01037), an adenosine A₁ agonist, CPA, and an adenosine A_{2A} agonist, CGS 21680. MA seeking induced by a MA priming injection or D₃ receptor stimulation was inhibited by a pretreatment with the adenosine A₁ agonist, CPA, but not the adenosine A_{2A} agonist, CGS 21680.

Conclusions: These results demonstrate the sufficiency of dopamine D₃ receptors to reinstate MA seeking that is inhibited when combined with adenosine A₁ receptor stimulation.

Keywords

Relapse; Psychostimulant; Purine; Adenosine receptor; Dopamine receptor

Corresponding author: Ryan K. Bachtell, Ph.D., Department of Psychology and Neuroscience, University of Colorado, 2860 Wilderness Place, Boulder, CO 80301; Phone: (303) 735-1012 Fax: (303) 492-2967; Ryan.Bachtell@Colorado.edu.

Conflict of interest:

The authors declare that there are no conflicts of interest.

Introduction

Methamphetamine (MA) is a highly addictive drug and the number of MA users in the United States continues to rise, along with MA-related hospitalizations and deaths (NIDA 2013). MA users are at a higher risk of relapse compared to other psychostimulant users, even after long periods of abstinence (Copeland and Sorensen 2001; Hartz et al. 2001). Despite the prevalence of MA addiction and its high abuse liability, the pharmacological mechanisms of MA seeking and relapse are not well understood. Further knowledge of these mechanisms would be beneficial in creating pharmacological treatments for MA abuse.

MA amplifies dopamine (DA) neurotransmission in the mesocorticolimbic system, which is comprised of neuronal projections originating in the ventral tegmental area and terminating in forebrain regions, such as the nucleus accumbens (NAc). Under normal physiological conditions, DA is packaged into vesicles through the vesicular monoamine transporter, released into the synapse, and recycled back into the presynaptic neuron via the dopamine transporter (DAT). MA potently reverses the actions of **the** vesicular monoamine transporter elevating cytoplasmic concentrations of DA (Brown et al. 2000; Volz et al. 2007). Non-vesicular cytoplasmic DA is subsequently transported from the cytoplasm into the synaptic cleft via MA-induced reverse action of the DAT (Fleckenstein et al. 1997). This rapid escalation of DA and the failure of DAT to transport DA into the presynaptic neuron prolongs DA receptor stimulation.

It is clear that DA receptor stimulation is important in the behavioral effects of MA, although stimulation of DA receptor subtypes produces differential effects on MA-induced behavior. DA D₁, D₂, and D₃ receptor subtype antagonists inhibit MA-induced locomotion, behavioral sensitization and conditioned place preference (Ujike et al. 1989; Bardo et al. 1999). The reinforcing properties of MA appear to be dependent on DA D₁ and D₃ receptors, but not DA D₂ receptors. DA D₁ and D₃ receptor antagonism, for example, disrupts MA-reinforced responding, whereas D₂ receptor antagonism has little effect (Brennan et al. 2009; Carati and Schenk 2011; Chen et al. 2014). DA D₁ and D₃ receptor antagonism also inhibits reinstatement of MA seeking in extinguished rats (Higley et al. 2011b, a; Carati and Schenk 2011; Chen et al. 2014). These studies utilizing subtype-selective antagonist approaches suggest that DA receptor subtypes differentially contribute to MA-induced locomotion, place preference, self-administration, and reinstatement of MA seeking. These antagonist approaches test the necessity of DA receptor subtypes, however, the sufficiency of DA receptor subtype stimulation to induce MA seeking is unknown. Therefore, one of the goals of these studies was to test the hypothesis that DA D₂-like receptor subtype stimulation **is** sufficient to induce MA reinstatement of drug seeking.

Adenosine is a negative neuromodulator of DA neurotransmission that has received attention due to its ability to inhibit psychostimulant behaviors (Golembiowska and Zylewska 1998; Filip et al. 2006, 2012; Bachtell and Self 2009; O'Neill et al. 2012; Hobson et al. 2013; Kavanagh et al. 2015; Wydra et al. 2015; Chesworth et al. 2016). Adenosine is a nucleoside that is found throughout the brain. Phasic increases in adenosine arise from increased neuronal metabolic activity and co-release of adenosine triphosphate (ATP) that is packaged in neurotransmitter vesicles (White 1977; Fredholm et al. 1982; Cass et al. 1987). For

example, DA is co-released with ATP that is subsequently metabolized into adenosine in the synapse. In the striatal areas, postsynaptic adenosine A₁ receptors co-localize with DA D₁ and D₃ receptors, while postsynaptic adenosine A_{2A} receptors co-localize with D₂ receptors, and have antagonizing effects on cellular functioning (Schiffmann et al. 1991; Svenningsson et al. 1999). Postsynaptic adenosine receptor stimulation provides a natural attenuation in DA receptor transmission through the formation of heteromeric receptor complexes and opposing intracellular signaling. This is thought to be a protective mechanism that may be absent following MA administration when nonvesicular DA is released. The striatum also contains presynaptic adenosine A₁ and A_{2A} receptors that are largely expressed on glutamate terminals (Ciruela et al. 2006; Quiroz et al. 2009). Stimulation of presynaptic adenosine A₁ receptors inhibits glutamate release and thereby reduces excitatory drive on striatal output pathways (Barrie and Nicholls 1993; Ciruela et al. 2006). Stimulation of presynaptic adenosine A_{2A} receptors, on the other hand, facilitates glutamate release and excitation of striatal output pathways (Popoli et al. 1995; Marchi et al. 2002; Rodrigues et al. 2005; Shen et al. 2013; Matsumoto et al. 2014). Therefore, pre- and postsynaptic adenosine receptors within the striatal areas intricately regulate the output from the striatal areas to influence behavior. Prior studies have demonstrated differential effects of adenosine receptor subtype stimulation on MA-induced locomotor activity, conditioned place preference and MA self-administration with adenosine A₁ receptor stimulation having the most potent inhibitory effects (Kavanagh et al. 2015). **We hypothesized that adenosine receptor stimulation attenuates MA seeking in a subtype-selective manner, consistent with previous observations.**

Materials and Methods

Animals

Male Sprague-Dawley rats (Envigo, Indianapolis, IN USA) initially weighing 275–325g were single-housed in polycarbonate “shoebox” cages (48.26cm × 26.67cm × 20.32cm) on a standard light-dark cycle (lights on at 0700h, lights off at 1900h) in a climate-controlled vivarium. **Rats were single housed to prolong catheter patency.** Rats had *ad libitum* food (Envigo, Teklad 2918) and water throughout the experimental procedures unless otherwise specified. Experimental procedures began at least 1-week after arrival and were conducted during the light period (1400h-1900h). All procedures were in accordance with the guidelines established by the National Institutes of Health and approved by the Institutional Animal Care and Use Committee at the University of Colorado Boulder.

Drugs

Methamphetamine hydrochloride, the DA D₂-like agonist, Quinpirole ((-)-quinpirole hydrochloride), the selective DA D₃ agonist, 7-OH-DPAT ((±)-7-Hydroxy-DPAT hydrobromide), and the selective adenosine A₁ receptor agonist, CPA (N6-Cyclopentyladenosine) were obtained from Sigma-Aldrich (St. Louis, MO). The selective DA D₂ agonist, sumanirole ((*R*)-5,6-Dihydro-5-(methylamino)-4*H*-imidazo[4,5,1-*ij*]quinolin-2(1*H*)-one (2*Z*)-2-butenedioate), the selective DA D₄ agonist, A412997 (*N*-(3-Methylphenyl)-4-(2-pyridinyl)-1-piperidineacetamide), the selective DA D₃ receptor antagonist, SB-77011A (*N*-[*trans*-4-[2-(6-Cyano-3,4-dihydro-2(1*H*)-

isoquinoliny]ethyl]cyclohexyl]-4-quinolinecarboxamide dihydrochloride) and PG01037 (*N*-[(2*E*)-4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]-2-buten-1-yl]-4-(2-pyridyl)-benzamide dihydrochloride), the selective adenosine A_{2A} receptor agonist, CGS 21680 (4-[2-[[6-Amino-9-(*N*-ethyl-*b*-D-ribofuranuronamidosyl)-9H-purin-2-yl]amino]ethyl]benzenepropanoic acid hydrochloride) were purchased from Tocris Bioscience (Bristol, UK). The relative selectivity of the chosen drugs for each dopamine D₂-like receptor subtype has been evaluated in prior studies (Levesque et al. 1992; Seeman and Van Tol 1994; Burris et al. 1995; Reavill et al. 2000; McCall et al. 2005; Moreland et al. 2005; Grundt et al. 2007). All drugs were diluted in sterile-filtered physiological (0.9%) saline except SB-77011A and PG01037 that were dissolved in 0.5% Tween-80, and 7-OH-DPAT that was dissolved in 50% DMSO.

Methamphetamine self-administration, extinction and reinstatement procedures

All self-administration and reinstatement testing was performed in operant conditioning chambers (Med-Associates, St. Albans, VT, USA) with two response levers and an infusion pump. To facilitate MA self-administration, rats were food restricted and trained to lever press for sucrose pellets on a fixed-ratio 1 (FR1) schedule of reinforcement. **Food was removed the night prior to the sucrose pellet self-administration session resulting in approximately 18 hours of deprivation and less than 5% loss of body weight. After successfully reaching the criterion (100 sucrose pellet deliveries in one session), rats were fed *ad libitum* for at least 1 day prior to surgical implantation of an intrajugular catheter. Approximately 90% of rats met the criterion within one session and all rats met this criterion within 3 sessions.** Following catheter implantation, animals were allowed 5 days of recovery in their home cage before experimental procedures began. During this time, catheters were flushed daily with 0.1 ml saline (0.26 mg/ml gentamicin and 20 U/ml heparin). After recovery, animals were allowed to self-administer intravenous MA (0.1 mg/kg/100μl infusion) on an FR1 schedule of reinforcement in 2-hour sessions for 10 consecutive days (Kitamura et al. 2006; Kavanagh et al. 2015). Methamphetamine injections were delivered over a 5-second period concurrent with a light cue over the drug-paired lever, followed by a 15-second timeout period **when** the house light remained off and lever presses had no consequence. Responses on the inactive lever produced no consequences. Following a 24-hour forced **abstinence** in the home cage, animals underwent 6 daily 2-hour extinction sessions, where responses to the lever previously paired with MA injections during self-administration and the inactive lever were recorded but resulted in no drug or cue delivery. Reinstatement of MA seeking was conducted in a 2-phase reinstatement test session (Bachtell and Self 2009; O'Neill et al. 2012) with each reinstatement session consisting of a 2-hour extinction phase (intended to eliminate spontaneous recovery and minimize baseline responding that may confound pharmacologically-induced drug seeking) and a 2-hour reinstatement phase that was initiated by systemic administration of a pharmacological agent (see below). Conditions during the reinstatement phase were identical to extinction (e.g. no drug/cue delivery or timeout).

Experiment 1: Effect of dopamine D₂-like receptor stimulation on reinstatement of methamphetamine seeking

The sufficiency of DA D₂-like receptor subtype (D₂, D₃, and D₄) stimulation to induce MA seeking was tested by administering the DA D₂-like agonist, quinpirole (0.1, 0.3, 1.0 mg/kg s.c.), the DA D₂ receptor agonist, sumanirole (0.3, 1.0, 10.0 mg/kg, i.p.), the DA D₃ receptor agonist, 7-OH-DPAT (0.3, 1.0, 10.0 mg/kg, i.p.), the DA D₄ receptor agonist, A412997 (0.3, 1.0, 10.0 mg/kg, i.p.), or vehicle 5 minutes prior to the reinstatement test session. Separate cohorts of rats were used to test each DA agonist. Repeated testing was conducted for each agonist with rats receiving a maximum of 3 reinstatement tests in a randomized order. One of the tests occurred under the vehicle conditions to provide a baseline of drug-seeking for each rat. **The other two tests evaluated the effects of two distinct DA agonist doses.** All agonist doses were not administered to each rat due to concerns of residual testing and weakening of reinstatement responding over repeated trials. An extinction session was conducted between each reinstatement test to minimize spontaneous recovery and residual effects from the previous reinstatement session. The doses and timing of injections were determined by previous studies examining the behavioral effects of DA agonists on reinstatement of drug seeking (Self et al. 1996; Graham et al. 2007).

Experiment 2: Effect of dopamine D₃ receptor antagonist on quinpirole-primed MA seeking

The combined effects of dopamine D₃ receptor antagonism and the D₂-like agonist, quinpirole, were tested on MA seeking by administering 0.3 mg/kg (s.c.) quinpirole, the dose producing maximal MA seeking (Fig. 2), in combination with the dopamine D₃ antagonist, SB-77011A (0, 6.0 and 12.0 mg/kg i.p.) or PG01037 (10 mg/kg, i.p.). Separate cohorts were used to test the effects of each antagonist. Vehicle, SB-77011A or PG01037 was administered 30 minutes prior to the quinpirole priming injection. The doses and timing of injections were determined by previous studies examining the effects of DA D₃ antagonists on reinstatement of MA seeking (Higley et al. 2011b, a).

Experiment 3: Effect of adenosine A₁ and adenosine A_{2A} receptor agonists on MA seeking induced by a MA priming injection

Two replicate cohorts were trained to self-administer MA and extinguished as described above. The effects of adenosine A₁ or A_{2A} stimulation on MA-primed drug seeking was tested in both cohorts by administering a pretreatment with the adenosine A₁ agonist, CPA (0.03, 0.1 mg/kg i.p.), the adenosine A_{2A} agonist, CGS 21680 (0.01, 0.03 mg/kg i.p.), or saline vehicle 5 minutes prior to MA (1.0 mg/kg i.p.). Each rat was tested repeatedly and received a maximum of 3 reinstatement tests in a randomized order. One of the tests occurred under the vehicle conditions to provide a baseline of drug-seeking for each rat. **The other two tests evaluated the effects of two distinct adenosine agonist doses.** The doses and timing of injections were determined by previous studies examining MA-primed drug seeking and the behavioral effects of adenosine agonists in a drug reinstatement model (Bachtell and Self 2009; Carati and Schenk 2011; Higley et al. 2011a; O'Neill et al. 2012; Hobson et al. 2013).

Experiment 4: Effect of adenosine A₁ and adenosine A_{2A} receptor agonists on MA seeking induced by dopamine D₂-like stimulation

The effects of adenosine A₁ or A_{2A} stimulation on quinpirole-primed and 7-OH-DPAT-primed MA seeking were tested in several cohorts by administering a pretreatment with adenosine A₁ agonist, CPA (0.03, 0.1 mg/kg i.p.), the adenosine A_{2A} agonist, CGS 21680 (0.01, 0.03 mg/kg i.p.), or saline vehicle 5 minutes prior to either quinpirole (0.3 mg/kg i.p.) or 7-OH-DPAT (1.0 mg/kg i.p.). Replicate cohorts were tested for quinpirole-primed MA seeking. Each rat was tested repeatedly and received a maximum of 3 reinstatement tests in a randomized order. One of the tests occurred under the vehicle conditions to provide a baseline of drug-seeking for each rat. **The other two tests evaluated the effects of two distinct adenosine agonist doses. The effects of adenosine A₁ or A_{2A} stimulation on 1.0 mg/kg (i.p.) 7-OH-DPAT-primed MA seeking was tested in a separate cohort of rats. Three separate reinstatement tests were conducted in randomized order to evaluate the following three conditions: Veh/7-OH-DPAT, 0.1 mg/kg (i.p.) CPA/7-OH-DPAT, 0.03 mg/kg (i.p.) CGS 21680/7-OH-DPAT.** The doses and timing of injections were determined by previous studies examining the behavioral effects of DA and adenosine agonists on reinstatement of drug seeking (Bachtell and Self 2009; O'Neill et al. 2012; Hobson et al. 2013).

Data Analysis

Lever responding during reinstatement testing was analyzed using a two-way mixed design ANOVA with the lever as the within-subject factor and treatment (drug dose or drug combination) as the between-subjects factor. In experiments 3 and 4, a common control group (Veh/MA and Veh/Quinpirole) was used **for analysis** since all animals were **tested** under identical vehicle pretreatment conditions. Significant interactions were followed by simple main effects analyses (one-way ANOVA) and Tukey's multiple comparison test. Statistical significance was set to $p < 0.05$ for all statistical tests.

Results

Methamphetamine self-administration and extinction

To illustrate the overall characteristics of the self-administration and extinction procedures used in all of the experiments, data from the cohort of animals tested for quinpirole-induced MA seeking in Experiment 1 are shown in Figure 1. Here, the average number of drug-paired and inactive lever responses as well as the average number of MA infusions over 10 MA self-administration sessions and 6 extinction sessions are plotted. Self-administration and extinction patterns observed for all experiments are detailed in Table 1.

Experiment 1: Stimulation of dopamine D₂-like receptors induces reinstatement of MA seeking

In this series of experiments, we assessed whether stimulation of DA D₂-like receptors was sufficient to induce reinstatement of MA seeking. Administration of the pan DA D₂-like agonist, quinpirole, was sufficient to induce reinstatement of MA seeking in a dose-dependent manner (Figure 2a). There was a significant interaction ($F_{3,36} = 4.08$, $p < 0.05$),

as well as main effects of quinpirole dose ($F_{3,36} = 4.97$, $p < 0.01$) and lever ($F_{1,36} = 27.30$, $p < 0.0001$). Post-hoc analysis revealed that both 0.3 mg/kg ($p < 0.01$) and 1.0 mg/kg quinpirole ($p < 0.01$) significantly increased drug-paired lever responding compared to vehicle and inactive lever responding. To further understand the role of specific DA D₂-like receptor subtypes in MA seeking, we assessed several agonists having preferential selectivity for the three DA D₂-like receptor subtypes. The DA D₂ receptor selective agonist, sumanirole, had no effect and produced no significant change compared with vehicle administration (Figure 2b). The DA D₃ receptor selective agonist, 7-OH-DPAT, significantly increased reinstatement of MA seeking (Figure 2c) as revealed by a significant interaction ($F_{3,39} = 9.68$, $p < 0.0001$) as well as significant main effects of 7-OH-DPAT dose ($F_{3,39} = 9.49$, $p < 0.0001$) and lever ($F_{1,39} = 19.40$, $p < 0.0001$). Post-hoc analysis revealed that only 1.0 mg/kg 7-OH-DPAT ($p < 0.001$) significantly increased drug-paired lever responding compared to vehicle and inactive lever responding. Lastly, we tested a DA D₄ receptor selective agonist, A412997, and observed no significant change in MA seeking compared with vehicle (Figure 2d). Together, these findings **demonstrate that stimulation** of the DA D₃ receptor subtype **induces** MA seeking, congruent with previous studies using antagonist approaches (Higley et al. 2011b, a).

Experiment 2: Blockade of dopamine D₃ receptors inhibits quinpirole-induced reinstatement of MA seeking

Because both the selective DA D₃ agonist and non-selective DA D₂-like agonist induced MA seeking, we sought to determine whether quinpirole-induced MA seeking could be inhibited by pretreatment with a DA D₃ receptor selective antagonist. As expected, the D₃ receptor antagonist, SB-77011A, significantly reduced MA seeking resulting from 0.3 mg/kg (s.c.) quinpirole administration (Fig. 3a). Significant main effects for SB-77011A dose ($F_{2,33} = 6.18$, $p < 0.001$), lever ($F_{1,33} = 36.31$, $p < 0.01$), and the interaction ($F_{2,33} = 3.93$, $p < 0.05$) were detected. Post-hoc analysis revealed that previously drug-paired lever responding was reduced following 12.0 mg/kg SB-77011A ($p < 0.01$) compared to vehicle and 6.0 mg/kg SB-77011A. Drug-paired lever responding remained significantly elevated compared with the inactive lever at all SB-77011A doses ($p < 0.05$). Similar results were observed with PG01037, another D₃ receptor selective antagonist (Fig. 3b). A significant interaction ($F_{1,22} = 8.31$, $p < 0.01$) and main effects for PG01037 ($F_{1,22} = 6.74$, $p < 0.05$) and lever ($F_{1,22} = 39.69$, $p < 0.001$) were detected. Here, a pretreatment with PG01037 significantly reduced drug-paired lever responding compared with vehicle ($p < 0.05$).

Experiment 3: Stimulation of adenosine A₁, but not A_{2A}, receptors attenuate MA-primed reinstatement of MA seeking

We tested whether stimulation of adenosine receptors would attenuate MA seeking induced by a systemic MA priming injection. Pretreatment with the adenosine A₁ receptor agonist, CPA, dose-dependently attenuated MA-induced seeking. There was a significant interaction ($F_{2,44} = 4.61$, $p < 0.05$), as well as significant main effects of lever ($F_{1,44} = 15.15$, $p < 0.001$) and CPA pretreatment ($F_{2,44} = 3.65$, $p < 0.05$). Figure 4 illustrates that a pretreatment with both doses of CPA (0.03 & 0.1 mg/kg) significantly reduced drug-paired lever responding ($p < 0.05$). Pretreatment with the adenosine A_{2A} receptor agonist, CGS 21680 produced a significant main effect of lever ($F_{1,48} = 34.39$, $p < 0.001$), but no statistical significance was

detected for the main effect of CGS 21680 pretreatment or the interaction. These results indicate that drug-paired lever responding remained increased compared to inactive responding regardless of CGS 21680 pretreatment (Fig. 4).

Experiment 4: Stimulation of adenosine A₁ and A_{2A} receptors differentially alter MA seeking induced by D₂-like receptor stimulation

Finally, we tested whether stimulation of adenosine receptors would attenuate MA seeking induced by 0.3 mg/kg quinpirole (s.c.) or 1.0 mg/kg (i.p.) 7-OH-DPAT. Pretreatment with either CPA or CGS 21680 dose-dependently reduced quinpirole-induced drug seeking (Fig. 5). For CPA, there was a significant interaction between lever and CPA pretreatment ($F_{2,75} = 4.93$, $p < 0.01$), as well as main effects of lever ($F_{1,75} = 30.96$, $p < 0.005$) and pretreatment ($F_{2,75} = 5.96$, $p < 0.05$). For CGS 21680, there was also a significant interaction between lever and CGS 21680 pretreatment ($F_{2,75} = 5.37$, $p < 0.01$), as well as main effects of lever ($F_{1,75} = 29.11$, $p < 0.001$) and pretreatment (CGS: $F_{2,75} = 5.93$, $p < 0.005$). Post-hoc analyses revealed a significant reduction in lever responding at both doses of CPA and CGS 21680 ($p < 0.05$). We also assessed the effects of adenosine receptor stimulation on MA seeking induced by the DA D₃ receptor agonist, 7-OH-DPAT. Here, we observed a selective inhibition of MA seeking by stimulation of the adenosine A₁ receptor and no effects of the adenosine A_{2A} receptor stimulation (Fig. 6). There was a significant interaction ($F_{2,17} = 4.86$, $p < 0.05$) and a main effect of lever ($F_{1,17} = 57.24$, $p < 0.001$), but not pretreatment ($F_{2,17} = 3.30$, $p = 0.06$). Post-hoc analyses revealed a significant reduction in 7-OH-DPAT-induced lever responding when CPA was administered as a pretreatment ($p < 0.01$).

Discussion

Our findings support a critical role for both DA D₂-like receptors and adenosine receptors in the reinstatement of extinguished MA responding. We found that the DA D₂-like receptor agonist, quinpirole, and the D₃ receptor agonist, 7-OH-DPAT, were sufficient to induce MA seeking. We also observed that stimulation of adenosine receptors altered MA seeking in a subtype-specific manner depending on how MA seeking was induced. In particular, only the adenosine A₁ receptor agonist, CPA, blocked MA and 7-OH-DPAT-induced drug seeking, while quinpirole-induced reinstatement of MA seeking was inhibited by stimulation of either adenosine A₁ (CPA) or A_{2A} (CGS 21680) receptors. Together, these findings demonstrate that DA D₂-like receptors are sufficient to induce MA seeking and adenosine receptors have the capacity to inhibit MA seeking.

Our findings are the first to report that DA D_{2/3} stimulation is sufficient to induce reinstatement of MA seeking. Previous studies have demonstrated the ability of DA D_{2/3} stimulation to induce reinstatement of cocaine and heroin seeking suggesting that DA D_{2/3} mechanisms are not specific for MA (Self et al. 1996; Khroyan et al. 2000; De Vries et al. 2002; Fuchs et al. 2002; Bachtell et al. 2005; Schmidt et al. 2006). In fact, using a reinstatement model of food seeking, dopamine D_{2/3} receptor stimulation was also sufficient to induce food seeking in the absence of priming and enhance food seeking induced by non-contingent delivery of food pellets (Duarte et al. 2003). These findings suggest that

stimulation of dopamine D_{2/3} receptors may generalize to other classes of drugs and non-drug behaviors.

Quinpirole is a non-selective DA D₂-like receptor agonist that has affinity at D₂, D₃, and D₄ receptor subtypes (Seeman and Van Tol 1994). Some studies have downplayed the role of the DA D₂ receptor subtype in MA-induced behavior. The non-selective D₂-like antagonist, eticlopride, failed to alter MA self-administration or MA seeking (Brennan et al. 2009; Carati and Schenk 2011). This is in contrast to evidence demonstrating that DA D₃ receptor antagonists inhibit MA-induced behavioral sensitization, conditioned place preference, self-administration, and cue- and MA-induced drug seeking (Higley et al. 2011b, a; Chen et al. 2014; Sun et al. 2016). The inconsistency between these antagonist studies is unclear but may reflect the marginally higher affinity of eticlopride for the D₂ receptor subtype and/or potential inverse agonist actions of eticlopride at the D₃ receptor (Hall et al. 1985; Griffon et al. 1996). Our findings that D₃, but not D₂- or D₄-selective stimulation was sufficient to induce reinstatement of MA seeking suggest that quinpirole's effects on MA seeking is likely due to stimulation at D₃ receptors. Further support for this notion comes from our evidence that DA D₃ receptor antagonism dose-dependently inhibits quinpirole-induced MA seeking. Drug discrimination studies demonstrate that dopamine D_{2/3} receptor stimulation generalizes to MA-discriminated responding, further suggesting that D_{2/3} receptor stimulation may recapitulate the subjective effects of MA administration and thereby contribute to D_{2/3}-induced MA seeking (Munzar and Goldberg 2000). Together with our findings, it appears that the sufficiency of DA D₂-like receptor stimulation to induce MA seeking results from stimulation of DA D₃ receptors, with little to no contribution of DA D₂ receptors.

The expression of DA D₂-like receptor subtypes throughout the brain differs among the family members, and this may play an important role in the observed effects on MA seeking. The D₂ receptor subtype is robustly expressed in the NAc and dorsal striatum, although it is also expressed throughout the brain where it functions as both a presynaptic autoreceptor and postsynaptic modulatory receptor (Sibley et al. 1993). The D₃ and D₄ receptors have more restricted expression in the brain where they also function as presynaptic autoreceptors and postsynaptic modulatory receptors. DA D₃ receptors are robustly expressed in the NAc, and DA D₄ receptors are highly enriched in the hippocampus and cortical brain regions (Sokoloff et al. 1990; Ariano et al. 1997; Gurevich and Joyce 1999). The enrichment of DA D₂ and D₃ receptors in the NAc and striatal regions is likely an important factor in their ability to induce MA seeking since DA receptor stimulation in the NAc has been strongly implicated in psychostimulant drug seeking (Self 2004; Bachtell et al. 2005; Schmidt et al. 2006).

Administration of the DA D₃ agonist, 7-OH-DPAT, produced an inverted U-shaped dose-response curve in MA seeking. MA seeking induced by the DA D₂-like agonist, quinpirole, displayed a similar pattern, although the descending limb was not as robust and likely reflects the limited dose range tested. Previous work has suggested that the synaptic localization of DA D_{2/3} receptors plays a role in the inverted U-shaped dose-response curves revealed on a variety of behaviors induced by DA D_{2/3} stimulation. For example, studies reveal locomotor suppression at low 7-OH-DPAT doses and behavioral

activation at higher 7-OH-DPAT doses with an increased prevalence of stereotypy at very high doses (Daly and Waddington 1993; Ahlenius and Salmi 1994; Ferrari and Giuliani 1995; Depoortere et al. 1996). There has been a long-standing hypothesis that the dose-related effects of 7-OH-DPAT, and other D₂-like agonists, on behavioral output are due to its differential impact on presynaptic DA D_{2/3} receptors at low doses and postsynaptic D_{2/3} receptors at higher doses. Thus, lower doses of 7-OH-DPAT are thought to stimulate presynaptic D_{2/3} receptors thereby decreasing locomotion and extracellular DA levels (Levant et al. 1996; De Boer et al. 1997), although this notion has been questioned by other studies (Svensson et al. 1994; Khroyan et al. 1995; Meyer 1996). Although the low baseline response rates prohibit the detection of a suppression in MA seeking in these studies, an absence of MA seeking is seen following the lowest 7-OH-DPAT dose tested. In contrast, MA seeking induced by the intermediate dose (1 mg/kg) of 7-OH-DPAT tested may have resulted from the stimulation of postsynaptic DA D_{2/3} receptors (Levant et al. 1996). However, as postsynaptic DA D_{2/3} receptor stimulation intensifies with higher doses, stereotyped behaviors may emerge and interfere with the MA seeking behavior. We see a modest increase in MA seeking at the highest 7-OH-DPAT dose tested, although this increase was not significantly different from vehicle. These findings are congruent with previous studies demonstrating an inverted U-shaped dose curve and may be suggestive of the stimulation of pre- versus postsynaptic DA D_{2/3} receptors.

We also found that stimulation of adenosine receptors reduced MA seeking in a subtype-selective manner. In particular, **stimulation of** adenosine A₁ receptors inhibited MA seeking induced by either MA or DA D₃ receptor stimulation. Stimulation of adenosine A_{2A} receptors, however, did not significantly reduce MA- or DA D₃-induced seeking. This adenosine receptor subtype selectivity is consistent with previous work showing that A₁, but not A_{2A}, receptor stimulation reduces MA self-administration (Kavanagh et al. 2015). Thus, it is surprising that the subtype-selectivity was lost when MA **seeking** was induced by the DA D₂-like agonist quinpirole. Regardless of the subtype specificity, our findings further support the role of adenosine receptor stimulation to inhibit behaviors regulated by enhanced DA neurotransmission (Golembiowska and Zylewska 1998; Filip et al. 2006, 2012; Bachtell and Self 2009; O'Neill et al. 2012; Hobson et al. 2013; Kavanagh et al. 2015; Wydra et al. 2015; Chesworth et al. 2016). Previous investigations into the behavioral regulation following DA and adenosine manipulations suggest that the striatal areas are a critical locus where postsynaptic adenosine receptor stimulation inhibits DA receptor transmission through the formation of heteromeric receptor complexes and opposing intracellular signaling in medium spiny neurons. (O'Neill et al. 2012; Hobson et al. 2012, 2013; Wydra et al. 2013; Borroto-Escuela et al. 2018). Adenosine receptor stimulation can also impact presynaptic neurotransmitter release. Stimulation of presynaptic adenosine A₁ receptors inhibits both dopamine and glutamate release while stimulation of presynaptic adenosine A_{2A} receptors facilitates glutamate release (Barrie and Nicholls 1993; Popoli et al. 1995; Marchi et al. 2002; Quarta et al. 2004; Rodrigues et al. 2005; Ciruela et al. 2006; Shen et al. 2013). Therefore, adenosine receptor stimulation could impact both DA and glutamate transmission within the striatal areas to influence MA seeking. Additional studies are necessary to fully assess the mechanisms associated with the ability of adenosine receptor stimulation to inhibit MA seeking.

Our findings suggest that DA D₃ receptor stimulation facilitates while adenosine A₁ receptors inhibit MA seeking, but these observations were only observed in male rats. Notable sex differences have been observed in a variety of MA-induced behaviors with females displaying more behavioral sensitivity. Compared with male rats, females generally show enhanced MA-induced locomotor activation, enhanced acquisition of MA self-administration and greater escalation in MA intake (Schindler et al. 2002; Roth and Carroll 2004; Milesi-Hallé et al. 2007; Reichel et al. 2012). Other studies demonstrate that MA seeking is also greater in females, although this may depend on the model used to test MA seeking (Cox et al. 2013; Ruda-Kucerova et al. 2015; Venniro et al. 2017). It is unclear whether the dopamine D₃ receptor or adenosine A₁ receptor stimulation would impact MA seeking similarly in females, but this could potentially be a fruitful area of research.

Together our findings suggest that stimulation of DA D₃ receptors is sufficient to reinstate MA seeking. Similar to previous work, adenosine receptor stimulation appears to inhibit MA seeking, with some A₁ receptor **selectivity** depending on how MA seeking was induced. We postulate that MA seeking results from stimulation of D₃ receptors expressed on D₁-containing medium spiny neurons in the striatal areas. Future studies should be aimed at disentangling the contributions of the DA D₁ and D₃ receptors specifically in the striatal areas and provide further characterization of the potential role for D₃ receptors in MA seeking behaviors. Finally, our results suggest that antagonism of DA D₁ and D₃ receptors, through either adenosine A₁ stimulation or another mechanism, could prove useful in developing a treatment for MA relapse.

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Figure 1. Methamphetamine self-administration and extinction in animals tested for quinpirole-induced reinstatement.

(a) Average number ($\pm$ SEM) of drug-paired lever responses, inactive lever responses and MA infusions (0.1 mg/kg/100 μ l infusion) for each 2-hour session over the 10-day self-administration procedure (n=17). (b) Average number of previously drug-paired and inactive lever presses ($\pm$ SEM) for each 2-hour session over the 6-day extinction phase (n=17). These data demonstrate the general response patterns during self-administration and extinction sessions across the various experiments and animals (see Table 1).

Figure 2. Dopamine D₂-like receptor stimulation induces reinstatement of extinguished MA responding in a subtype-specific manner.

(a) Subcutaneous administration of dopamine D₂-like agonist, quinpirole, significantly increased drug-paired lever responding following administration of 0.3 and 1.0 mg/kg (n=10/group). (b) Intraperitoneal administration of the dopamine D₂ specific agonist, sumanitrole, had no effect on the drug-paired lever responding at any of the doses tested (Vehicle, n=10; 0.1 mg/kg, n=10, 0.3 mg/kg, n=10; 1.0 mg/kg, n=9). (c) Intraperitoneal administration of the dopamine D₃-specific agonist, 7-OH-DPAT, significantly increased drug-paired lever responding at 1.0 mg/kg (Vehicle, n=16; 0.3 mg/kg, n=8, 1.0 mg/kg, n=12; 10.0 mg/kg, n=9). (d) Intraperitoneal administration of dopamine D₄ specific agonist, A412997, had no effect on the drug-paired lever responding at any of the doses tested (Vehicle, n=14; 1 mg/kg, n=14, 3 mg/kg, n=13). Data are presented as mean lever responses $\pm$ SEM. * Significant from vehicle treatment (Tukey's test, $p < 0.001$) # Significant from inactive lever responding (t-test, $p < 0.05$)

Figure 3. MA seeking induced by a dopamine D₂-like agonist is inhibited by dopamine D₃ receptor antagonism.

(a) Systemic administration of dopamine D₃ antagonist, SB-77011A (12.0 mg/kg, i.p.), inhibits 0.3 mg/kg (s.c.) quinpirole-induced MA seeking at drug-paired lever responding (n=12/group). (b) Systemic administration of dopamine D₃ antagonist, PG 01037 (10.0 mg/kg, i.p.), also inhibits 0.3 mg/kg (s.c.) quinpirole-induced MA seeking at drug-paired lever responding (n=12/group). Data are presented as mean lever responses $\pm$ SEM. * Significant from vehicle pretreatment (Tukey's test, $p < 0.05$); # Significant from inactive lever responding ($p < 0.05$).

Figure 4. MA seeking induced by a MA priming injection is inhibited by adenosine A₁, but not A_{2A}, receptor stimulation.

Intraperitoneal administration of the adenosine A₁ agonist, CPA (0.1 mg/kg), but not adenosine A_{2A} agonist, dose-dependently attenuates 1.0 mg/kg (i.p.) MA-induced drug-paired lever responding (Vehicle, n=26; 0.03 mg/kg CPA, n=12, 0.1 mg/kg CPA, n=9; 0.01 mg/kg CGS 21680, n=12, 0.03 mg/kg CGS 21680, n=13). Data are presented as mean lever responses $\pm$ SEM. * Significant from vehicle pretreatment (Tukey's test, $p < 0.05$); # Significant from inactive lever responding ($p < 0.05$).

Figure 5. MA seeking induced by dopamine D₂-like receptor stimulation is inhibited by adenosine A₁ and A_{2A} receptor stimulation.

Intraperitoneal administration of the adenosine A₁ agonist, CPA, and A_{2A} agonist, CGS 21680, significantly blunted 0.3 mg/kg (s.c.) quinpirole-induced drug-paired lever responding (Vehicle, n=37; 0.03 mg/kg CPA, n=17, 0.1 mg/kg CPA, n=24; 0.01 mg/kg CGS 21680, n=17, 0.03 mg/kg CGS 21680, n=24). Data are presented as mean lever responses $\pm$ SEM. * Significant from vehicle pretreatment (Tukey's test, $p < 0.05$); # Significant from inactive lever responding ($p < 0.05$).

Figure 6. MA seeking induced by dopamine D₃ receptor stimulation is inhibited by adenosine A₁, but not A_{2A}, receptor stimulation.

Intraperitoneal administration of the adenosine A₁ agonist, CPA (0.1 mg/kg), but not adenosine A_{2A} agonist, dose-dependently attenuates 1.0 mg/kg (i.p.) 7-OH-DPAT-induced drug-paired lever responding (n=10/group). Data are presented as mean lever responses $\pm$ SEM. * Significant from vehicle pretreatment (Tukey's test, $p < 0.05$); # Significant from inactive lever responding ($p < 0.05$).

Table 1.

Methamphetamine self-administration infusions and extinction responding across experiments

	SA/EXT Day1	SA Day 10 EXT Day 6
Experiment 1		
<i>Quinpirole-induced MA seeking (n = 17)</i>		
Self-Administration: Infusions	20.4 ± 2.7	30.9 ± 2.9
Extinction: Drug-paired lever responses	65.8 ± 5.9	17.9 ± 4.9
<i>Sumanitrole-induced MA seeking (n = 10)</i>		
Self-Administration: Infusions	14.4 ± 1.9	25.9 ± 4.0
Extinction: Drug-paired lever responses	56.3 ± 11.7	12.2 ± 2.3
<i>7-OH-DPAT-induced MA seeking (n = 17)</i>		
Self-Administration: Infusions	18.5 ± 4.4	24.4 ± 1.5
Extinction: Drug-paired lever responses	59.8 ± 6.4	16.7 ± 1.9
<i>A412997-induced MA seeking (n = 14)</i>		
Self-Administration: Infusions	16.9 ± 3.0	31.6 ± 6.2
Extinction: Drug-paired lever responses	46.0 ± 4.1	16.1 ± 2.2
Experiment 2		
<i>Dopamine D₃ antagonist (SB-01177) pretreatment on quinpirole-induced MA seeking (n = 18)</i>		
Self-Administration: Infusions	14.7 ± 2.0	27.6 ± 2.1
Extinction: Drug-paired lever responses	55.1 ± 5.4	11.5 ± 1.4
<i>Dopamine D₃ antagonist (PG01037) pretreatment on quinpirole-induced MA seeking (n = 15)</i>		
Self-Administration: Infusions	19.7 ± 4.3	40.9 ± 6.7
Extinction: Drug-paired lever responses	54.8 ± 8.4	18.5 ± 3.6
Experiment 3		
<i>Adenosine agonist pretreatment on MA-induced seeking (n = 26)</i>		
Self-Administration: Infusions	21.3 ± 6.3	28.4 ± 3.6
Extinction: Drug-paired lever responses	55.2 ± 5.2	21.2 ± 4.2
Experiment 4		
<i>Adenosine agonist pretreatment on quinpirole-induced MA seeking (n = 41)</i>		
Self-Administration: Infusions	17.0 ± 1.9	31.8 ± 3.6
Extinction: Drug-paired lever responses	65.8 ± 9.1	21.7 ± 3.7
<i>Adenosine agonist pretreatment on 7-OH-DPAT-induced MA seeking (n = 10)</i>		
Self-Administration: Infusions	12.9 ± 2.1	29.17 ± 1.7
Extinction: Drug-paired lever responses	56.8 ± 9.4	17.3 ± 2.4